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[CN/US]; 6054 Signal Flame Ct., Clarksville, MD 21029 (US). BAO, Jun [CN/US]; 16329 Sierra Ridge Way, Hacienda Heights, CA 91745 (US). MAO, Hua [CN/US]; 16329 Sierra Ridge Way, Hacienda Heights, CA 91745 (US). MA, Wenbin [CA/US]; 6502 Mallery Ct., Frederick, MD 21703 (US). LI, Lina [CN/US]; 20810 Amber Ridge Drive, Germantown, MD 20876 (US).

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(74) Agents: YE, Michael et al.; Andrews Kurth LLP, 1701 Pennsylvania Avenue, N.W. Suite 300, Washington, D.C. 20006 (US).

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(71) Applicant (*for all designated States except US*): **ALLIED BIOTECH, INC.** [US/US]; 10075 Tyler Place, #19, Ijamsville, MD 21754 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **LI, Zhuangwu**

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR DETECTING TELOMERASE ACTIVITY

Providing a reaction tube that contains a first reaction mixture having a first primer and deoxynucleoside triphosphates in a top portion of the tube, a second reaction mixture having a second primer and a DNA polymerase in a lower portion of the tube, and a layer of wax separating the first reaction mix from the second reaction mixture

102

Adding a biological sample to the first reaction mixture and incubating the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer

104



Elongating the extension product

106



Admixing the extension product with the second reaction mixture by melting the wax layer

108



Amplifying the extension product by real-time PCR

110



Quantifying the amplified extension product using a control template

112

(57) Abstract: A method for determining telomerase activity using primer extension followed with real time PCR quantification is disclosed. The method of the present invention provides a rapid, sensitive and accurate measurement for telomerase activity in a biological sample. In one embodiment, the method includes the steps of: adding the biological sample to a reaction tube containing a first reaction mixture having a first primer and nucleoside triphosphates, a second reaction mixture having a second primer and a DNA polymerase, and a wax layer that separates the first reaction mixture from the second reaction mixture; incubating the biological sample with the first reaction mixture; admixing the extension product with the second reaction mixture; amplifying and quantifying the extension product using real-time PCR and a control template. In another embodiment, the detection method includes an *in situ* primer extension step that allows the production of the extension product within an intact cell. In this embodiment, the extension product can be preserved under appropriate conditions for an extended time before the completion of the quantification step.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Rec'd PCT/PTO 16 MAY 2005
 Inte n Application No
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A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C12Q1/68 C12Q1/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>ELMORE ET AL: "Real-time quantitative analysis of telomerase activity in breast tumor specimens using a highly specific and sensitive fluorescent-based assay" DIAGNOSTIC MOLECULAR PATHOLOGY, vol. 11, no. 3, September 2002 (2002-09), pages 177-85, XP008029655 cited in the application the whole document</p> <p>-----</p> <p>-/-</p>	1-7, 15-18

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

2 June 2004

Date of mailing of the international search report

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Osborne, H

INTERNATIONAL SEARCH REPORT

Intel

Final Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HOU M ET AL: "Real-time quantitative telomeric repeat amplification protocol assay for the detection of telomerase activity" CLINICAL CHEMISTRY, vol. 47, no. 3, 2001, pages 519-24, XP002276809 cited in the application the whole document -----	1-7, 15-18
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Intel

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BURGER A M ET AL: "Inhibition of Telomerase Activity by Cisplatin in Human Testicular Cancer Cells" EUROPEAN JOURNAL OF CANCER, PERGAMON PRESS, OXFORD, GB, vol. 33, no. 4, April 1997 (1997-04), pages 638-644, XP004282569 ISSN: 0959-8049 the whole document -----	20

INTERNATIONAL SEARCH REPORT

Inte
onal application No.
PCT/US 03/35919

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 15-18 and partially 20

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7, 15-18 and partially 20

A single-tube *in vitro* assay method for quantitative determination of telomerase activity in a cell sample using real-time PCR, and application of the method for monitoring the effectiveness of treatment of a patient.

2. claims: 8-14, 19 and partially 20

An *in vivo* assay method for quantitative determination of telomerase activity in a cell involving real-time PCR, and application of the method for monitoring the effectiveness of treatment of a patient.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte
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